MTA1 Expression Correlates Significantly with Histologic Grade in Salivary Mucoepidermoid Carcinoma

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Abstract

Background: Metastasis associated protein-1 (MTA1) has been a recently identified as a unique gene playing important role in tumorigenesis and progression of cancer cells.
Objective: To evaluate MTA1 expression and its predictive value in determining histologic grade of salivary mucoepidermoid carcinoma (MEC).
Patients and Methods: MTA1 expression was evaluated by immunohistochemistry in paraffin-embedded tumor specimens blocks from 22 patients. Assessment of MTA1 immunostaining was achieved by counting the proportion of positively-stained tumor cells in 5 high power microscopic fields; and staining was analyzed in relation to clinicopathological variables.
Results: MTA1 show nuclear and cytoplasmic expression in varying intensity in 95% of cases. No significant correlation was found between MTA1expression and age, gender, site of the tumor ($p>0.05$). However, statistically significant correlation was found between MTA1expression and clinical stage, nodal involvement ($p=0.009$ and 0.007; respectively). Regarding histologic grade, high MTA1 level was significantly associated with grade of tumors categorized by Auclair and Brandwein systems (<0.001 and 0.009; respectively).
Conclusion:MTA1 expression significantly correlates with tumor grade and progression, and has a potential role in diagnosis and prediction of behavior in salivary MEC.
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Introduction

Salivary gland mucoepidermoid carcinoma (MEC) is clinically and pathologically, a relentless disease with different biologic behavior.1 MEC is the most common salivary gland carcinoma, which is approximately accounting for 5-15 of all salivary neoplasms and 30-35% of salivary gland malignancies[2].The histopathologic features of MEC are complex and largely based on the various criteria of grading systems; and generally these tumors are categorized tumor into low, intermediate, or high grade[3].In salivary gland carcinoma, the histologic grade ranks highly among the most important prognostic parameters, although grading systems still have no consensus concerning its accurate value for predicting outcome and tumor behavior[4,5].

Metastasis-associated protein-1 (MTA1), the basic member of the MTA family was originally identified via differential screening of the cDNA Library from rat metastatic breast tumors as an up regulated gene[6,7]. MTA1 overexpression was seen in various human cancers and shown to be involved in tumor invasion, and metastasis; regardless its nuclear and cytoplasmic expression[8,9]. In previous study, the investigators observed that MTA1 was overexpressed during neoplastic transformation, and it was inversely related to tumor progression[10]. Whereas, Dias (2013) found that nuclear overexpression of MTA1 is significantly correlated to the aggressive biologic behavior and metastasis in human prostate cancer[11]. Regarding tumor grading, elevated MTA1 expression was found to be closely associated with higher grade in human cancers[12]. However, some researchers found a negative correlation between MTA1expression and clinicopathologic features of salivary MEC[13,14]. The purpose of this study was to assess the correlation between MTA1 expression and the histologic grade of salivary MEC.

Patients and Methods

This study included 22 pretreatment formalin fixed, paraffin embedded salivary MEC that retrieved from the archives of the Department of Oral Diagnosis / College of Dentistry/ Baghdad University, and Department of specialized surgery in Al-Shaheed Ghazi Hospital in Baghdad. Clinical data concerning the age, gender, site of tumor and lymph node metastasis was obtained from patients' medical records for the period extending from 2009 to 2017. For all specimens, 4µm thick sections were prepared and stained with hematoxylin and eosin (H&E) stain to confirm the diagnosis. Clinicopathologic characteristics of the overall series are summarized in Table (1). The histological grade of tumors, in this study, was evaluated according to criteria of Auclair 1991), in addition to schemes proposed by Brandwein , (2001), which are approved by The WHO classification of tumors[15,16]. All the cases were staged according to the 7th edition of the American Joint Committee on Cancer (AJCC)[17].

Immunohistochemistry (IHC) and analysis

The sections in these series were deparaffinized in xylene, rehydrated through graded alcohols, immersed in 3% H2O2 for 20 min to inhibit endogenous peroxidase activity, and antigen retrieval performed using citrate buffer with PH=6. Nonspecific binding was blocked with 1% serum albumin at room temperature for 10 min, then the sections were incubated with anti-MTA1 rabbit-polyclonal antibody (1:500 dilution; Abcam, Cambridge, UK) overnight at 4°C in a humidified chamber. Negative controls were achieved by omitting the primary antibody. After washing with PBS, the tissue sections were incubated with biotin-free, anti-rabbit secondary antibody conjugated with horseradish peroxidase (HRP) for 15 min, and then stained with 3, 3-diaminobenzidine (DAB), counterstained with Mayer's hematoxylin, dehydrated and mounted.

Statistical analysis

The degree of IHC staining was separately evaluated by two pathologists who were blinded to the clinicopathologic information. MTA1 immunostaining was scored by calculating the proportion of positively stained tumor cells in 5 microscopic high power fields that reveal higher immunopositivity as follows: Score 0 (0-5% positive cells); Score 1 (6-25% positive cells); score 2 (26-50% positive cells); score 3 (51-75% positive cells) and Score 4 (≥ 76 positive cells). A Mann-Whitney test, Kruskal Wallis test, Chi-Square test, Spearman´s correlation coefficient test were used to compare the result between groups and the relation with clinical-pathological parameters such as patient age, gender, tumor site, metastasis to lymph nodes, tumor grade and clinical stage. We used the SPSS version 24 software to statistically analyze the data. P-values <0.05 were considered statistically significant in all cases.

Results

Representative images of MTA1 immunohistochemical expression in MEC are shown in Figure(1A-F).Positive immunostaining of MTA1 was observed in both nuclear and cytoplasmic compartments in (95%) of the MEC tissues. While, negative or weak MTA1stanining was found in the adjacent non-cancerous ductal epithelial tissues in the same section Figure(1A).

Correlation between MTA1 expression and the clinicopathological features of salivary MEC

As shown in Table(2), the relationship between MTA1 expression and the clinicopathological characteristics of all series of salivary MEC. There was no statistically significant correlation between MTA1 protein expression and clinicopathological features, such as age, gender and tumor site (P>0.05). However, the expression level of MTA1 protein was found to be significantly associated with clinical stage, showing a lower expression pattern in early-stage disease (I and II), and a stronger expression (median MTA1 score 4), in late stages (III and IV; P = 0.009). High MTA1 expression also was found to be significantly associated with positive nodal metastasis (p=0.007). Regarding both Auclair and Brandwein grading systems, the median MTA1 score was significantly higher among tumors with high grade (score-3) compared to those with low grade (median score=2), thus a statistically significant correlation was found between MTA1 overexpression and tumor grade categorized by Auclair and Brandwein systems (p value <0.001 and 0.009; respectively).

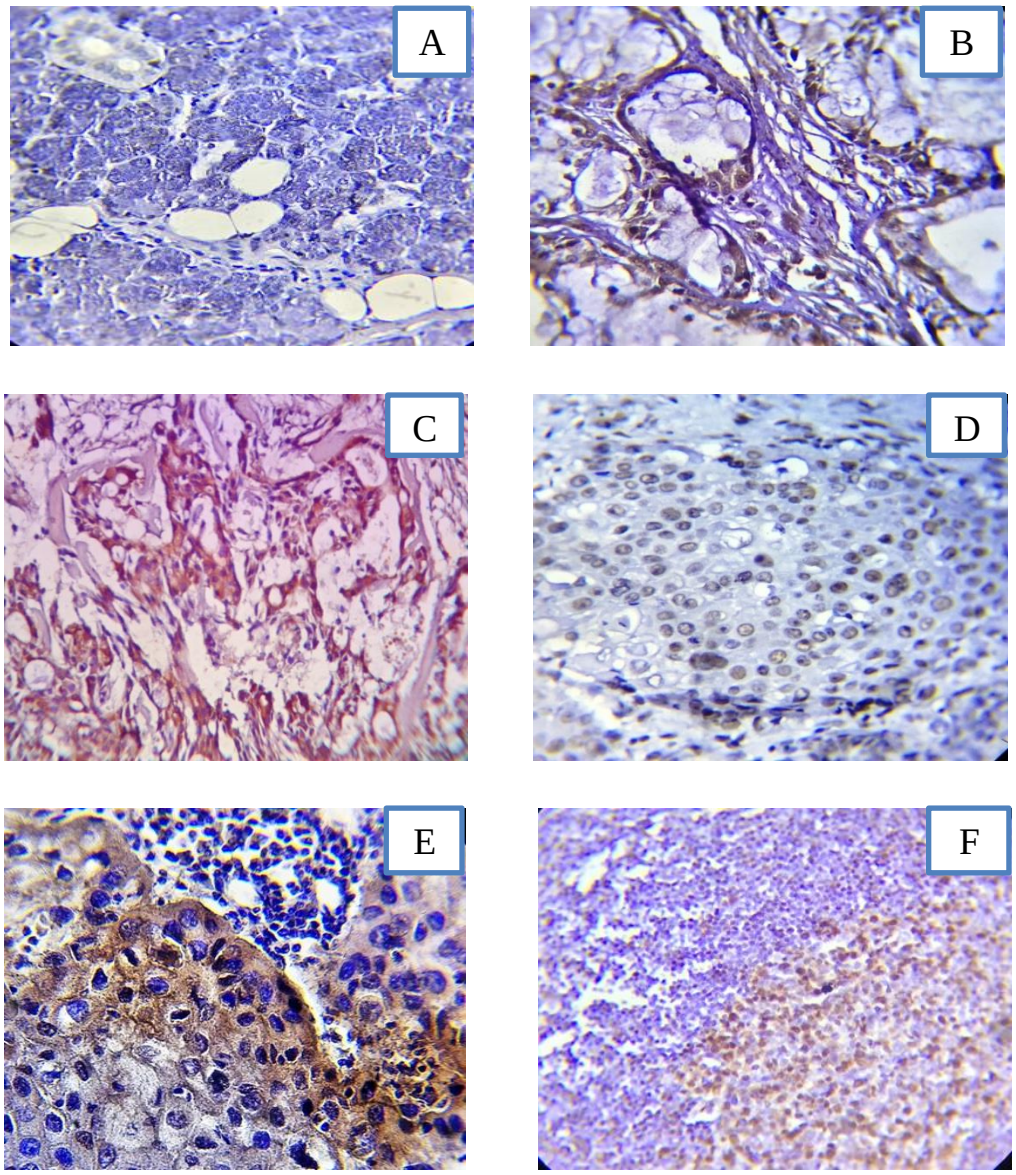

Figure (1): Immunohistochemical analysis of MTA1 protein in salivary mucoepidermoid carcinoma. (A) Negative staining in normal salivary ductal tissue (40x); (B) low grade tumor shows cystic components lined by mucous and intermediate cell: brown stain (40x); (C) MEC, with small islands of intermediate and epidermoid cell, mainly cytoplasmic expression (20x); (D) High grade MEC with nuclear MTA1expression (40x); (E) Solid tumor with sheets of anaplastic epidermoid cells with cytoplasmic expression (40x); (F) lymph node infiltrated with tumor cells expressing nuclear MTA1 protein (20x).

Table (1): Clinical-pathological parameters of the overall series.

Parameters	Total		
	No.	(%)	
Age group (years)			
<40	5	22.7	Mean ±SD of (45.6± 13.1)
>40	17	77.3	
Gender			
Female	12	54.5	
Male	10	45.4	
Site			
Major	11	50	
Minor	11	50	
Clinical stage			
I	9	40.9	
II	7	31.8	
III	3	13.6	
IV	3	13.6	
Lymph node status			
-ve	16	72.7	
+ve	6	27.3	
Histologic grade			
Auclair system			
Low	15	68.1	
High	7	31.8	
Brandwein system			
I	6	27.2	
II	6	27.2	
III	10	45.4	

Table (2): Correlation between MTA1 expression and the clinicopathologic features.

Parameters	No.of cases	MTA1 scores					Median MTA score	p-value
		0	I	II	III	IV		
Age group (years)								
<40	5	0	1	3	1	0	2	0.15
>40	17	1	3	6	5	2	3	[NS]
Gender								
Male	10	0	1	4	3	2	3	0.10
Female	12	1	3	5	3	0	2	[NS]
Site								
Minor	11	0	1	6	3	1	2	0.45
Major	11	1	3	3	3	1	2	[NS]
Clinical Stage								
I-II	16	1	4	8	3	0	2	0.009*
III-IV	6	0	0	1	3	2	4	
Lymph node metastasis								
N0	16	1	4	8	3	0	2	
N1	3	0	0	0	1	2	4	0.007 *
N2	3	0	0	1	2	0	3	
Auclair grading system								
Low	15	1	4	9	1	0	2	<0.001*
High	7	0	0	0	5	2	3	
Brandwein grading system								
Low	6	1	2	3	0	0	2	0.009*
Intermediate	6	0	2	3	1	0	2	
High	10	0	0	3	5	2	3	

*significant relation ($p<0.05$); [NS] non- significant ($p>0.05$)

Discussion

Histologic grade of salivary gland carcinomas is a significant predictor of tumor aggressiveness and patient's outcome. However, the sheer diversity of tumor differentiation and the rarity of these tumors pose challenges to invent highly predictive grading schemes. Grading of MEC is not without flaws, one clear deficiency in all systems, particularly the point based schemes, was the difficulty in application[18,19]. Thus, it is necessary to find a new biomarkers that specifically predict tumor aggressiveness and behavior. MTA1 gene is a regulating factor that mediated cell signaling pathway and chromosomes remodeling, and also has transcriptional activity, so it is implicated in tumor progression and invasion of metastatic epithelial cells[20,21]. In the present study, we evaluated MTA1 expression in salivary MEC, and there was no statistically significant correlation between MTA1 level

and clinical findings, such as patient age, gender and tumor site. This finding was in agreement with previous studies[22,23]. In this study, also we observed that MTA1expression was significantly correlated with clinical stage and lymph node metastasis. Our finding was in line with other researches which were indicated, by immunohistochemical analysis that MTA1 protein expression is significantly correlated with aggressive tumor progression, and positive nodal status in head and neck cancer[24,25]. However, our results were not in conformity with findings from Andishehtadbir, (2016) who found that MTA1 protein expression had no significant statistical relation with clinical stage, lymph node status and metastasis of salivary MEC which may be attributable to an insufficient number of cases, in which a small size of sample was associated with tumor progression and positive lymph node metastasis[14].On the other hand, we observed that a significant cohort of our series showed nuclear MTA1 expression compared to cytoplasmic one, which suggest the invasive and metastatic potential of these tumors[22].Earlier investigations stated that MTA1s, a short version of cytoplasmic MTA1 may bind to estrogen receptor-alpha (ER-α) and inhibit its nuclear function by non-genomic activity of (ER-α) that occurs in cytoplasm of cancer cells. Thus, this may rationalize the aggressive behavior of these tumors[22,26]. Regarding tumor grading, we found a significant up-regulation of MTA1 expression in higher grade tumors classified according to both Auclair and Brandwein system ($p< 0.001$ and 0.009, respectively). In this context, we found a significant strong positive linear correlation between MTA1 score ($r=0.7$) and Brandwein tumor grade. This result suggests a strong association of MTA1 expression with the progression of MEC. In contrast to our findings, Andishehtadbir (2016), found no correlation between MTA1 expression and histologic grade of salivary MEC, although high MTA1 expression was seen in cases with advanced tumor size and clinical stage. The possible cause behind this disagreement is that, in our analysis we have approved a standardized grading systems in tumor classification, with focus on more subjective criteria, compared to previous study which was built on a descriptive morphologic and histologic features[14].

Conclusion

MTA1 expression significantly correlates with histologic grade and progression of salivary MEC and may be a valuable biomarker in diagnosis and predicting tumor behavior.

References

[1] Pires FR, Almeida OP, de Araújo VC, Kowalski LP. Prognostic factors in head and neck mucoepidermoid carcinoma. Arch Otolaryngol Head Neck Surg 2004; 130: 174-180.

[2] Luna MA. Salivary mucoepidermoid carcinoma: revisited. Adv Anat Pathol 2006; 13: 293-307.

[3] Barnes L. Surgical Pathology Of Head and Neck: Diseases of the Salivary Glands, Third Edition.USA:2009; p. 506.

[4] Seethala RR. Histologic Grading and Prognostic Biomarkers in Salivary Gland Carcinomas. Adv Anat Pathol. 2011; 18: 29-45.

[5] Ahmed O. and Al-Azzawi L. Salivary mucoepidermoid carcinoma: Auclair vs Brandwein grading system (histopathological comparative study). 2017; 977.

[6] Toh Y, Pencil SD, Nicolson GL. A novel candidate metastasis-associated gene, mta1, differentially expressed in highly metastatic mammary adenocarcinoma cell lines. cDNA cloning, expression, and protein analyses. J Biol Chem 1994; 269:22958–63.

[7] Toh Y and Nicolson GL. The role of the MTA family and their encoded proteins in human cancers: molecular functions and clinical implications. Clin Exp Metastasis 2009; 26:215-27.

[8] Nicolson GL, Nawa A, Toh Y, Taniguchi S, Nishimori K, Moustafa A. Tumor metastasis-associated human MTA1 gene and its MTA1 protein product: role in epithelial cancer cell invasion, proliferation and nuclear regulation. Clinical & experimental metastasis 2003;20(1):19-24.

[9] Sen N, Gui B, and Kumar R. Role of MTA1 in Cancer Progression and Metastasis. Cancer Metastasis Rev. 2014; ; 33(4): 879–89.-9.

[10] Yu, C. H., Chen, H. H.,Wang, J. T., Liu, B. Y., Wang, Y. P., Sun, A. , Kuo, R. C. , Chiang, C. P. Nuclear expression of metastasis-associated protein 1 (MTA1) is inversely related to progression of oral squamous cell carcinoma in Taiwan. J Dent Sci 2007; 2:1.

[11]Dias, S.J., et al.. Nuclear MTA1 overexpression is associated with aggressive prostate cancer, recurrence and metastasis in African Americans. Sci. Rep. 2013; 3: 2331.

[12] Jang k, Paik S, Chung H, Oh Y and Kong G. MTA1 overexpression correlates significantly with tumor grade and angiogenesis in human breast cancers. Cancer Sci. 2006; 97 (5): 375.

[13]Andisheh-Tadbir A, Ashraf MJ, Khademi B, Ahmadi Sh. Clinical implication of CD166 expression in salivary gland tumor. Tumor Biology 2015; 36(4): 2793-9.

[14]Andisheh-Tadbir A, Dehghani-Nazhvani A, Ashraf MJ, Khademi B, Mirhadi H, Torabi-Ardekani S. MTA1 Expression in Benign and Malignant Salivary gland Tumors. Iranian Journal of Otorhinolaryngology, 2016; 28(1):51-59.

[15]Auclair PL, Goode RK. Ellis GL Mucoepidermoid carcinoma of intraoral salivary glands: evaluation and application of grading criteria in 143 cases. Cancer 1992;69:2021-2030.

[16]Brandwein MS, Ivanov K, et al. Mucoepidermoid carcinoma. A clinicopathological study of 80 patients with special reference to histological grading. Am J Pathol 2001;25:835–45.

[17] Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A: AJCC Cancer Staging Manual. 7th edition. New York: Springer; 2010.

[18] Seethala RR. An update on grading of salivary gland carcinomas. Head Neck Pathol. 2009; 3: 69–77.

[19] Seethala RR HA, Bennett A, Arrosi AA, Dvison JM, Krasinkas AM, Hunt JL. Reproducibility of grading in salivary gland mucoepidermoid carcinoma and correlation with outcome: does system really matter? Mod. Pathol. 2008; 2; Abstract 1105.

[20]Liu, J., Haijuan Wang , Changzhi Huang , Haili Qian. Subcellular localization of MTA proteins in normal and cancer cells. Cancer Metastasis Rev 2014a ; 33, 843–856.

[21]Liu, J., Haijuan Wang , Changzhi Huang , Haili Qian. The subcellular distribution and function of MTA1 in cancer differentiation. Oncotarget 2014b; 5: 5153- 64.

[22] Li W F, Liu N, Xue R X, He Q M, Chen M, Jiang N, Sun Y, Zeng J, Liu L Z and Jun MNuclear overexpression of metastasis-associated protein 1 correlates significantly with poor survival in nasopharyngeal carcinoma. Journal of Translational Medicine 2012, 10:78.

[23]Liu T, Yang M, Yang S, Ge T, Gu L, Lou G. Metastasis-associated protein 1 is a novel marker predicting survival and lymph nodes metastasis in cervical cancer. Hum Pathol. 2013;44(10):2275–81.

[24] Kawasaki, S. Yanamoto, I. Yoshitomi, S Yamada, A Mizuno: Overexpression of metastasis-associated MTA1 in oral squamous cell carcinomas: correlation with metastasis and invasion. Int. J. Oral Maxillofac. Surg. 2008; 37: 1039–1046.

[25] Yuan T, Zhang H, Liu B, Zhang Q, Liang Y, Zheng R, Deng J, Zhang X. Expression of MTA1 in nasopharyngeal carcinoma and its correlation with prognosis. Med Oncol. 2014; 31:330.

[26]Kumar R, Wang RA, Mazumdar A, Talukder AH, Mandal M, Yang Z, et al. A naturally occurring MTA1 variant sequesters oestrogen receptor-alpha in the cytoplasm. Nature. 2002;418(6898):654-7.